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Nanostructured styrenic co-polymers containing glucopyranosyl residues and their functionalization

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ABSTRACT

Sugar-based co-polymers with saccharidic units in stable cyclic form and nanometric morphologies stabilized through crosslinking, adaptable through specific functionalizations to biochemical interaction studies with copper-containing amine oxidases, were synthesized from appropriate monomers and macromonomers. The most promising nanospherical co-polymer obtained, containing β-D-glucopyranosidic units, was employed in functionalization reactions with the help of model molecules, achieving useful transformations mainly at the 6-position and to a minor extent at the 2-position of the saccharidic system.

1. Introduction

Nanostructured polymeric systems find increasing applications in various biomedical fields[1] such as biosensors, tissue engineering and drug delivery systems. In particular, hydrophilic nanostructured glycopolymers with saccharidic moieties at the particle surface extending towards the aqueous medium are attractive for their possible interactions with proteins[2] or cells[3] and utility in drug delivery.[3]

As a part of our ongoing interest in copper amine oxidases (CAOs),[4–9] a diverse class of enzymes[10] that control important cellular processes such as cell proliferation and crosslinking of elastin and collagen, we have recently reported the synthesis of crosslinked nanostructured gluconamidic vinyl co-polymers and their functionalization by O-alkylation with benzaldehydic residues,[11] as models for more complex fuctionalizations aimed to localized studies of angiogenesis and cell proliferation.

The free radical polymerization (FRP) of sugar-based vinyl monomers, successfully employed in the synthesis of glycopolymers[12–15] for simplicity of operation, solvent compatibility and tolerance to many monomer functionalities, offers an interesting variety of experimental polymerization conditions such as in bulk, in solution and in multiphase systems including suspension, emulsion, dispersion and precipitation polymerizations. Precipitation polymerization, which avoids the use of additives such as stabilizers or emulsifiers, has been successfully exploited for the synthesis of molecularly imprinted microspheres,[16,17a,b] nanoparticles[17a,b] and nanostructured gluconamidic vinyl co-polymers.[11]

In this work we report the synthesis and functionalization of new sugar-based co-polymers having nanostructured morphologies stabilized through crosslinking and bearing saccharidic units in stable cyclic glucopyranosidic form (structures **a** and **b**, Chart 1). This enables an alternative ordering of the hydroxyl groups influencing adhesion of proteins without altering the effect of the added bioactive functions, allowing the production of materials, which can be exploited in biomedical research based on the interaction with copper-containing amine oxidases.

We achieved the preparation of the target sugar-based co-polymers in the form of nanostructured systems through the

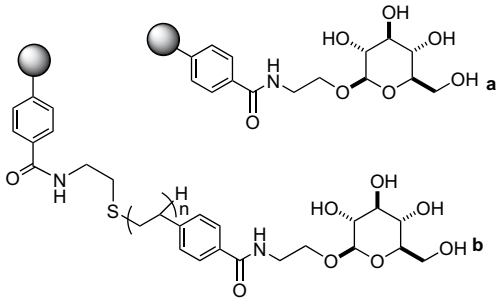

Chart 1.

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radical precipitation polymerization of new glucopyranosidic monomers and macromonomers, and examined their functionalization with the help of model molecules.

2. Results and discussion

2.1. Monomers and macromonomers

The new monomers **5**, **6**, **7** and **8**, with the polymerizable function linked to C-1 of the sugar moiety in stable cyclic structure, were synthesized from **1**[18] (Scheme 1) through the known intermediates **2**,[19a–c] **3**[19b,20] and **4**.[21] The peracetylated monomers **5** and **7** were obtained in the form of stable spectroscopically characterized crystalline solids. Monomer **6**, obtained from the deprotection of **5**, was a low melting solid, difficult to manipulate due to its hygroscopicity and tendency to polymerize, so it was not employed in polymerization experiments. Monomer **8**, obtained in high yield by deacetylation of **7**, was a stable, lightly hygroscopic, white solid. The ^{1}H, ^{13}C NMR and DEPT-135 spectra of **8** confirmed its structure and the β-stereochemistry of the anomeric carbon. In particular the ^{1}H NMR spectrum recorded in DMSO-d_6 showed peaks for NH, secondary OH groups at C-2, C-3 and C-4, and primary OH at C-6 and H-1 as well separated signals (Experimental).

The macromonomers **Mac6** and **Mac8** were obtained by parallel syntheses from peracetylated monomers **5** and **7** through radical oligomerization to form **Olig5** and **Olig7** in the presence of 2-aminoethanethiol hydrochloride,[22] functionalization with 4-vinylbenzoyl chloride[23] to form **Mac5** and **Mac7** and final deacetylation. Scheme 2 shows the conversion of **5** and **7** into **Mac6** and **Mac8**, respectively.

The number average molecular weight values (M_n) of **Olig5** and **Olig7** determined by acidimetric titration of the amino end group were 1312 and 1580, respectively, close to the theoretical values of 1450 and 1678 calculated for a degree of oligomerization of three.

The styrenic **Mac5** and **Mac7** discoloured Br_2 in CCl_4 and aqueous $KMnO_4$ and gave a negative ninhydrin test, thus proving the presence of the double bond and the absence of the amino group. They both proved insoluble in hexane, Et_2O, water and soluble in benzene, $CHCl_3$, CH_2Cl_2, acetone, dioxane, pyridine, THF, DMF, EtOH.

The deacetylation of **Mac5** and **Mac7** with MeONa in MeOH/dioxane was monitored by observing the disappearance of the strong absorptions at 1755 and 1230 cm^{-1} of the ester groups and the appearance of very strong hydroxyl bands at 3410 cm^{-1} in the FTIR spectra of **Mac6** and **Mac8**, which proved soluble only in water, DMSO, DMF and partially soluble under heating in MeOH and EtOH.

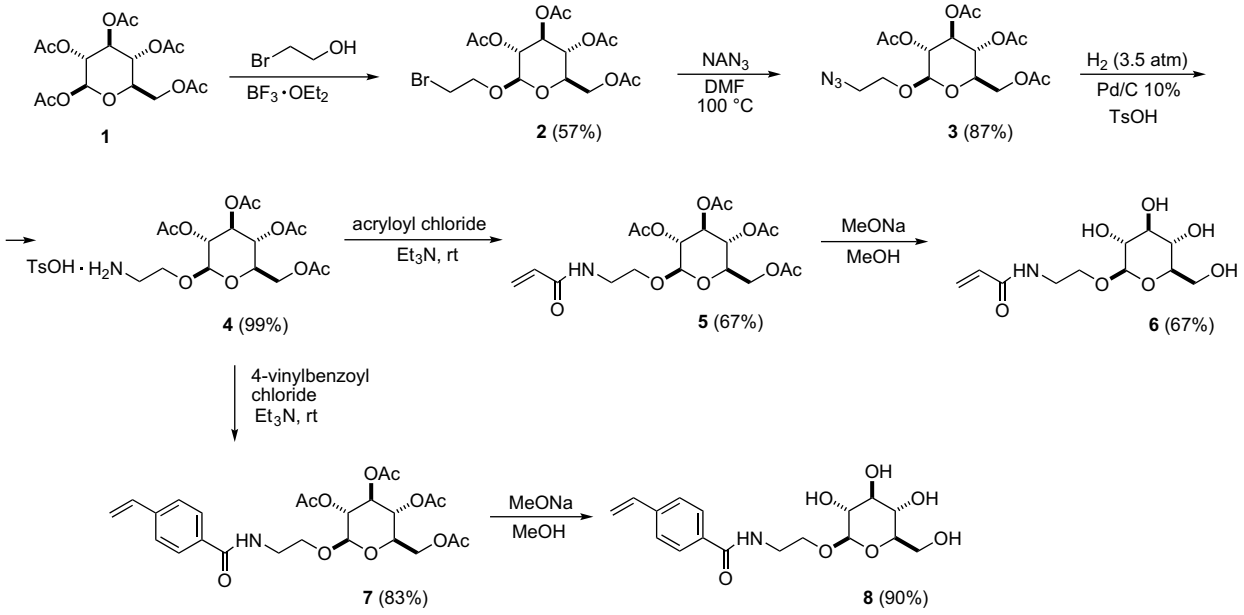
2.2. Precipitation co-polymerizations

Hydroxyl-rich **Mac6**, **Mac8** and **8**, were co-polymerized with styrene or crosslinkers by radical precipitation polymerization in the presence of appropriate solvents. The monomer feed molar composition for the macromonomers was calculated on the basis of an average degree of oligomerization of three, corresponding to a nominal molecular weight of 1039 for **Mac6** and 1267 for **Mac8**.

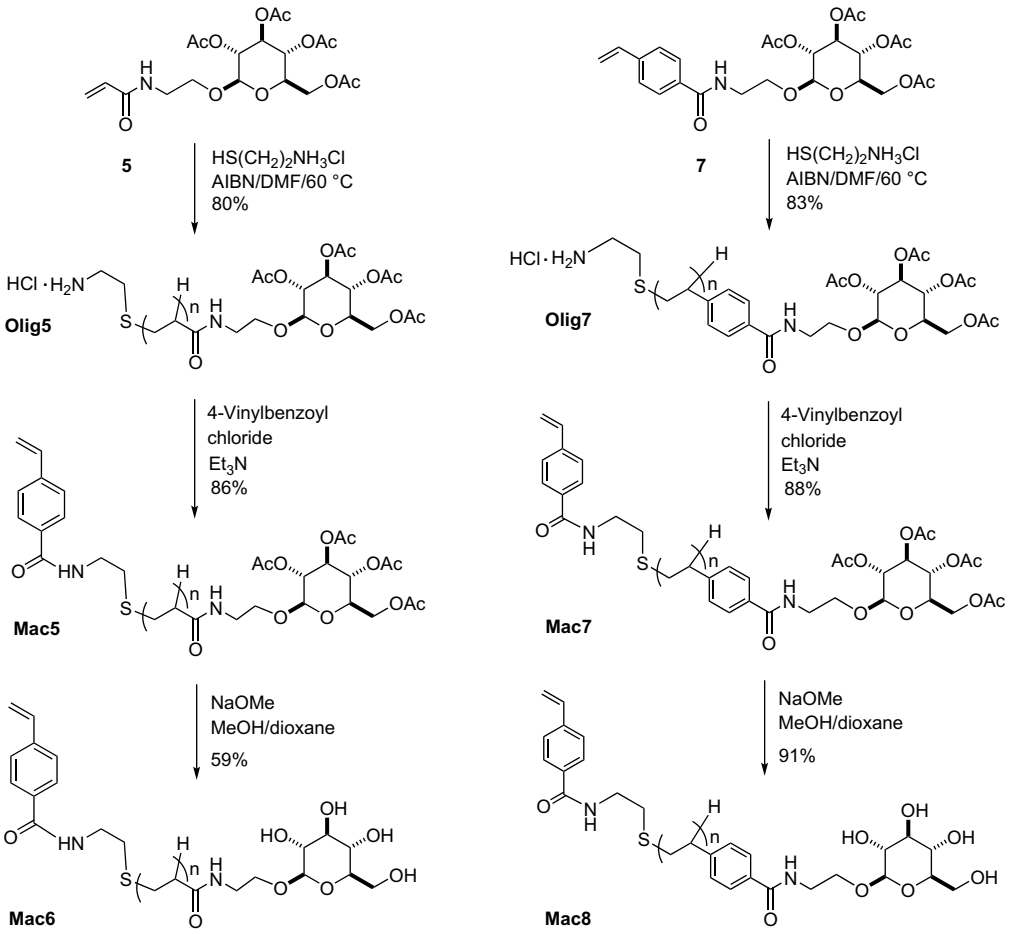
Mac6 and **Mac8** were co-polymerized at 60 °C with styrene in mixtures H_2O/EtOH (1/1.5–1.6 v/v, depending on macromonomer feed molar composition in the range 0.17–0.80). **Mac6** was co-polymerized with 1,4-divinylbenzene (DVB) as crosslinker at 70 °C (in H_2O/EtOH=1:1.9) to afford co-polymers, which under observation with Scanning Electron Microscopy (SEM) showed irregular morphologies of little interest.

Mac8 afforded co-polymers with peculiar lamellar structures when co-polymerized as reported in Table 1 with DVB (**CM8-1**), or with DVB and styrene (**CM8-2**), or with other crosslinkers such as ethylenebisacrylamide (EBA) (**CM8-3**), or ethyleneglycol dimethacrylate (EGDM) (**CM8-4**). Figure 1a and b shows two typical morphologies of the prepared co-polymers. The investigation of these lamellar structures was beyond the aim of this work and it was not pursued. Nevertheless, it must be observed that the co-polymers **CM8** prepared from the macromonomer **Mac8** are expected to have 'comb-like' structure and amphiphilic nature, two properties that in other glycoconjugated polystyrenes[24] give rise to ordered conformations, stable in aqueous urea solution, where they form cylindrical 'bottle-brush' micelles.

Monomer **8**, subjected to precipitation co-polymerization with DVB in MeOH, afforded spherically shaped co-polymers (**C8**) (Table 1) with average diameter of 589±89 nm (Fig. 2), which were chosen for functionalization experiments.



Scheme 1.



Scheme 2.

2.3. Functionalization of co-polymers obtained from 8

The study of co-polymer functionalization through deprotonation of the saccharidic hydroxyl groups with strong bases and reaction with alkyl chlorides to form stable ether bridges required the synthesis of suitable model molecules to ascertain which hydroxyl groups were involved. Model compound **10** based on the molecular structure of monomer **8** was synthesized according to Scheme 3 from the intermediate **9** obtained from **4** or **1**, with the latter route being preferred to the former.

Table 1
Typical co-polymerization data of **Mac8** and **8** at 70 °C

Monomer mg (mmol)	Comonomer g (mmol)	Crosslinker mg (%)	AIBN mg (%)	Solvent mL	Time (h)	Polymer mg (%)
Mac8	—	DVB		H_2O/EtOH	24	**CM8-1**
350		53.0	20.0	1/1.7		110.5
(0.22)[a]		(12.1)	(4.6)	(72)		(27.4)
Mac8	STY	DVB		H_2O/EtOH	24	**CM8-2**
100	2.05	5.1	16.7	1/1.5		11.4
(0.08)[a]	(19.7)	(5.0)	(5.1)	(17.5)		(10.6)
Mac8	—	EBA		H_2O/EtOH	74	**CM8-3**
100		4.0	5.2	1/1.14		11.5
(0.08)[a]		(4.0)	(5.2)	(15)		(11.1)
Mac8	—	EGDM		H_2O/EtOH	48	**CM8-4**
100		4.0	5.2	1/1.4		12.2
(0.08)[a]		(4.0)	(5.2)	(17)		(11.7)
8	—	DVB		MeOH	70	**C8**
213		8.5	4.3			56.9
(0.60)		(3.2)	(1.9)	(43)		(25.9)

DVB=divinylbenzene; STY=styrene; EBA=ethylenebisacrylammide; EGDM=ethyleneglycol dimethacrylate.

[a] Calculated from the reported nominal molecular weight of 1267 corresponding to a nominal loading of 0.789 mmol/g of saccharidic units.

DEPT-135 and HETCOR experiments allowed the assignment of all the carbon signals of the saccharidic portion of **10**. In addition, the ^{1}H NMR spectrum of **10** in DMSO-d_6 showed signals for secondary OH groups at C-4, C-3 and C-2, primary OH at C-6 and amidic NH (Experimental). These were very useful for revealing the position and multiplicity of the alkylation reaction.

Model compound **10** was deprotonated with NaH in less than stoichiometric ratio (Table 2) to limit multisubstitution followed by alkylation with model chlorides carrying readily detectable aldehydic groups like the very reactive allylic chloride **11**[11] or the primary alkyl chloride **12**.[11] This afforded, under different reaction conditions, products of O-alkylation mono or disubstituted at the positions 6, 2 and 4.

The reaction of **10** with NaH (0.9 equiv) then with stoichiometric (or excess) allyl chloride **11** in DMF at 24 °C (Table 2) gave mixtures of products, which by preparative layer chromatography (PLC) afforded some unreacted **10**, unresolvable mixtures of **13** and **14** in ratios ranging from 1.44/1 to 2.25/1, small quantities of **15** and dialkylation products, and non-saccharidic by-products. PLCs of pooled fractions from different runs of mono and dialkylated mixtures allowed the isolation of very small amounts of **15** and **16**, however sufficient for their characterization.

Mono and double alkylation products were established by observing the ratios of integrals of the NH signals with respect to the

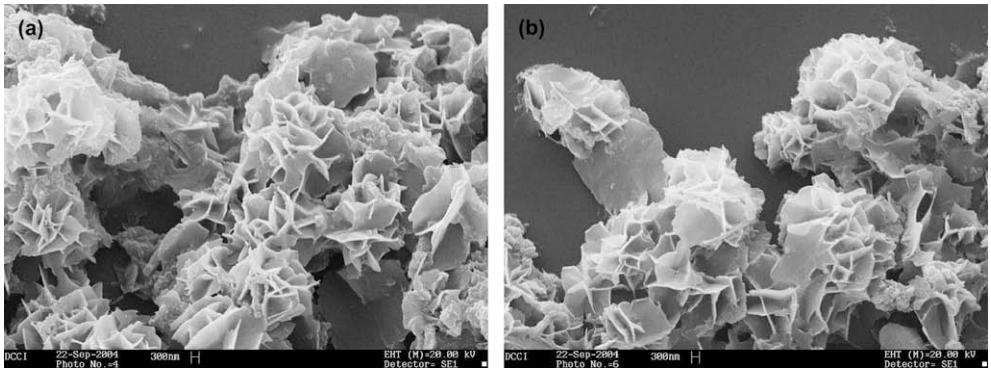


Figure 1. SEM images of the co-polymers **CM8-3** (a), **CM8-4** (b).

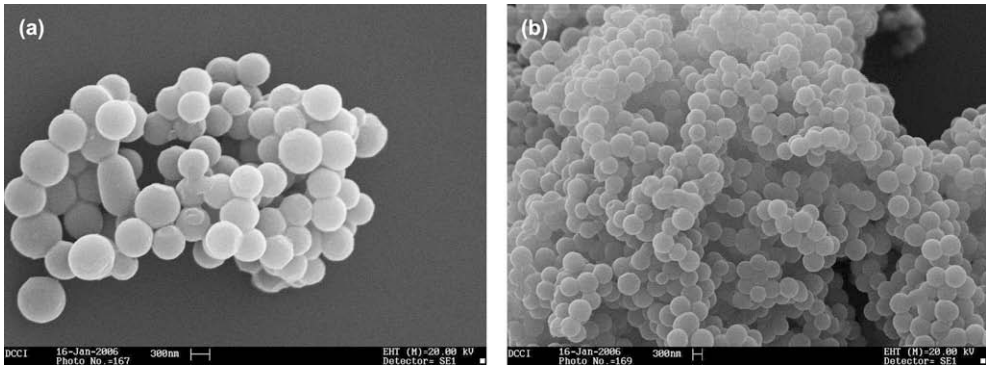
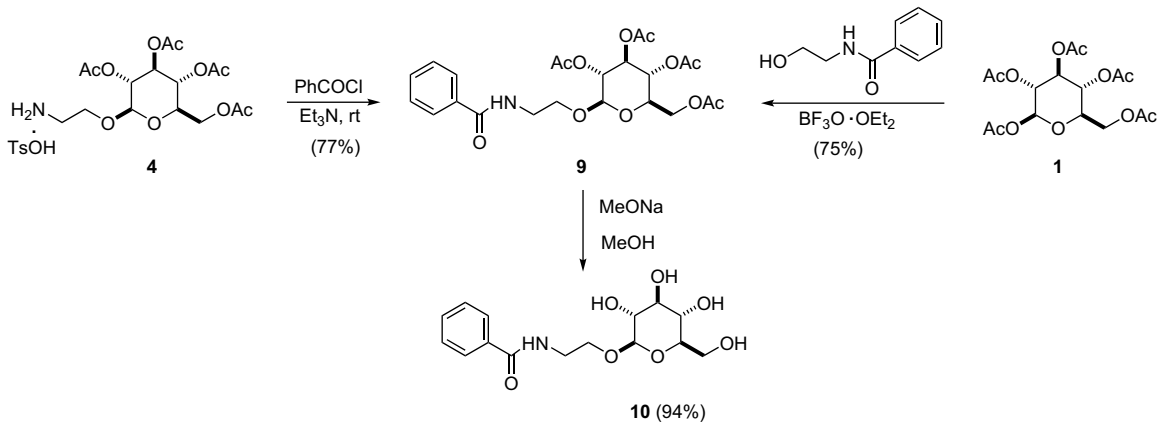


Figure 2. SEM images of the co-polymer **C8**.



Scheme 3.

aldehydic ones in the ^{1}H NMR spectra (1:1 for monoalkylation and 1:2 for double alkylation, respectively).

The attributions of the structures **13**–**16** were achieved by comparing the DEPT-135 ^{13}C NMR spectra of the saccharidic portion of model **10** and alkylation products, and by looking for the expected jump of about 7–9 ppm associated with the conversion of hydroxyl carbon to alkoxyl carbon (Fig. 3, Chart 2).

The ^{13}C NMR spectrum of **16** confirmed the hydroxyl groups at C-6 and C-2 in **10** as the preferred reactive sites, with the disappearance of the signals at 62.00 ppm (C-6) and 74.40 (C-2) and the appearance of signals at 70.81 and 81.81 ppm, respectively being observed.

The alkylation of **10** with **11** carried out at 0 °C gave rise to suppression of double substitution and increase of the **13**/**14** ratio up to 5.88.

The addition of dibenzo-18-crown-6 to the reacting mixture for the alkylation of **10** with **11** to avoid possible preferential intramolecular complexation[25] and to promote the reactivity of naked alkoxy ions eliminated double substitution and lowered the reaction selectivity slightly, reducing the **13**/**14** ratio.

The GC–MS analysis of the non-saccharidic portion of the various runs revealed, besides unreacted **11** (11–34%), the presence of 4-(4-hydroxy-2-butenyloxy)benzaldehyde (3–5%), *N*-(2-hydroxyethyl)-benzamide (about 11%) and largely variable percentages of 4-hydroxybenzaldehyde under different reaction conditions, evidencing the formation of spurious products.

A reactivity test of **11** treated at 24 °C for 240 h with MeONa in MeOH gave only the expected methyl ether by chlorine substitution.

Table 2
Reaction data of the alkylation of **10** with **11** and **12** in DMF

Run	**10** mg (mmol)	Chloride	Molar ratio **10**/NaH/chloride	*t* °C	Time H	A mg (%)	**16** mg (%)	C-6/C-2 alkylation ratio
1	89.4 (0.273)	**11**	1/0.9/1	24	24	21.5 (17.9)	4.8 (2.6)	2.25 (**13**/**14**)[a]
2	95.8 (0.293)	**11**	1/0.9/1	24	72	24.0 (18.4)	4.7 (2.4)	2.10 (**13**/**14**)[a]
3	101.2 (0.309)	**11**	1/0.9/3	24	24	25.0 (17.9)	7.8 (3.7)	1.44 (**13**/**14**)[a]
4	96.4 (0.295)	**11**	1/0.9/1	0	144	23.3 (17.5)	—	5.88 (**13**/**14**)[a]
5	100.7 (0.308)	**11**	1/0.9/1[b]	24	8	30.8 (22.1)	—	1.50 (**13**/**14**)[a]
6	87.2 (0.266)	**11**	1/0.9/1[b]	24	24	24.7 (20.5)	—	1.70 (**13**/**14**)[a]
7	91.9 (0.281)	**12**	1/0.9/1	24	99	7.9 (6.2)	—	5.08 (**17**/**18**)[c]
8	96.9 (0.296)	**12**	1/0.9/1	80	4	12.1 (9.0)	—	1.50 (**17**/**18**)[c]

A=mixture of monoalkylated products.
[a] From the –CH=CH– signals.
[b] In the presence of 18-dibenzo crown ether (0.9 equiv).
[c] From the anomeric CH signals.

A stability test of a mixture **13**/**14** treated with NaH in DMF at 24 °C for 48 h produced both *N*-(2-hydroxyethyl)benzamide and 4-hydroxybenzaldehyde as shown by GC–MS.

Side reactions and the formation of by-products contributed to make the overall alkylation yields rather modest, nevertheless the obtained yields, if transferred to co-polymers, would correspond to functionalization in the range 0.4–0.6 mmol/g, sufficiently good for the planned bioactive co-polymers.

The reaction of **10** with NaH, then with **12**, carried out in a similar way as with **11**, at 24 °C gave only mono-substituted products at positions 6 and 2 producing an unresolvable mixture of **17** and **18** (Table 2). This is in agreement with the lower reactivity of the primary alkyl chloride, with lower yields for longer reaction time being observed.

The increase of reaction temperature to 80 °C never gave rise to the formation of dialkylation products and promoted a modest increase of yields of monoalkylation with the formation of an undetermined small amount of **19** alkylated at C-4. Since the GC–MS of the non-saccharidic portion of the mixture revealed the presence of 4-(2-butenyloxy)benzaldehyde indicating alteration of **12**, prolonged reaction times were not explored further.

In the strongly basic conditions of the functionalization of **10** with **11** or **12**, undesired reactions of aldehydic carbonyls with hydroxyl groups were never observed.

Samples of the nanospherical co-polymer **C8** were subjected to treatment with NaH then with allyl chloride **11**. It was concluded that **11** was more reactive and attractive than **12** as a model for appropriate bioactive reagents. The co-polymers underwent functionalization parallelling those of the model molecule **10**. After the removal of low molecular weight aldehydes and spurious alkylation products by careful washing and centrifugation, the functionalized co-polymers gave positive Schiff's tests as shown by the fuchsia colour of variable intensity developed on the surface of the solid co-polymers, not removable by washing.

3. Conclusions

With the view of attaining new research tools in the field of copper-containing amine oxidases based on new saccharidic nanostructured co-polymers functionalized to make them capable of specific interaction with the enzyme active site, we synthesized monomers **5**, **6**, **7** and **8**.

Monomer **6** was discarded for its poor *physical* properties. Peracetylated monomers **5** and **7** were transformed into the macromonomers **Mac6** and **Mac8**, respectively.

Mac6, **Mac8** and **8** were subjected to precipitation co-polymerization in the presence of crosslinkers affording polymeric materials with different morphologies. Co-polymers from **Mac6**

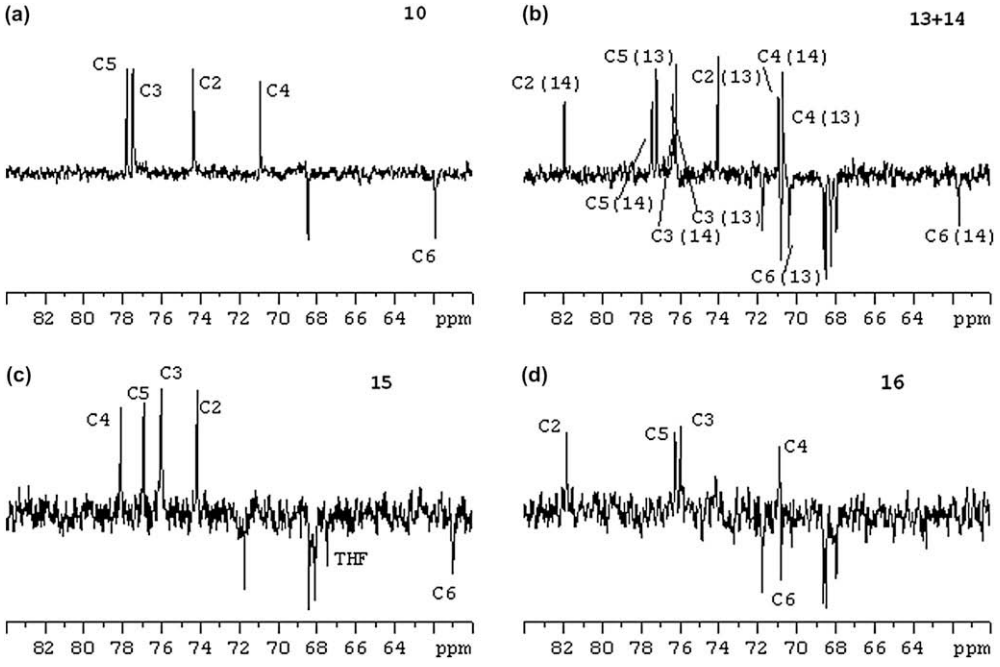


Figure 3. DEPT-135 spectra of the saccharidic portion of **10** (a), mixture **13**+**14** (b), **15** (c) and **16** (d).

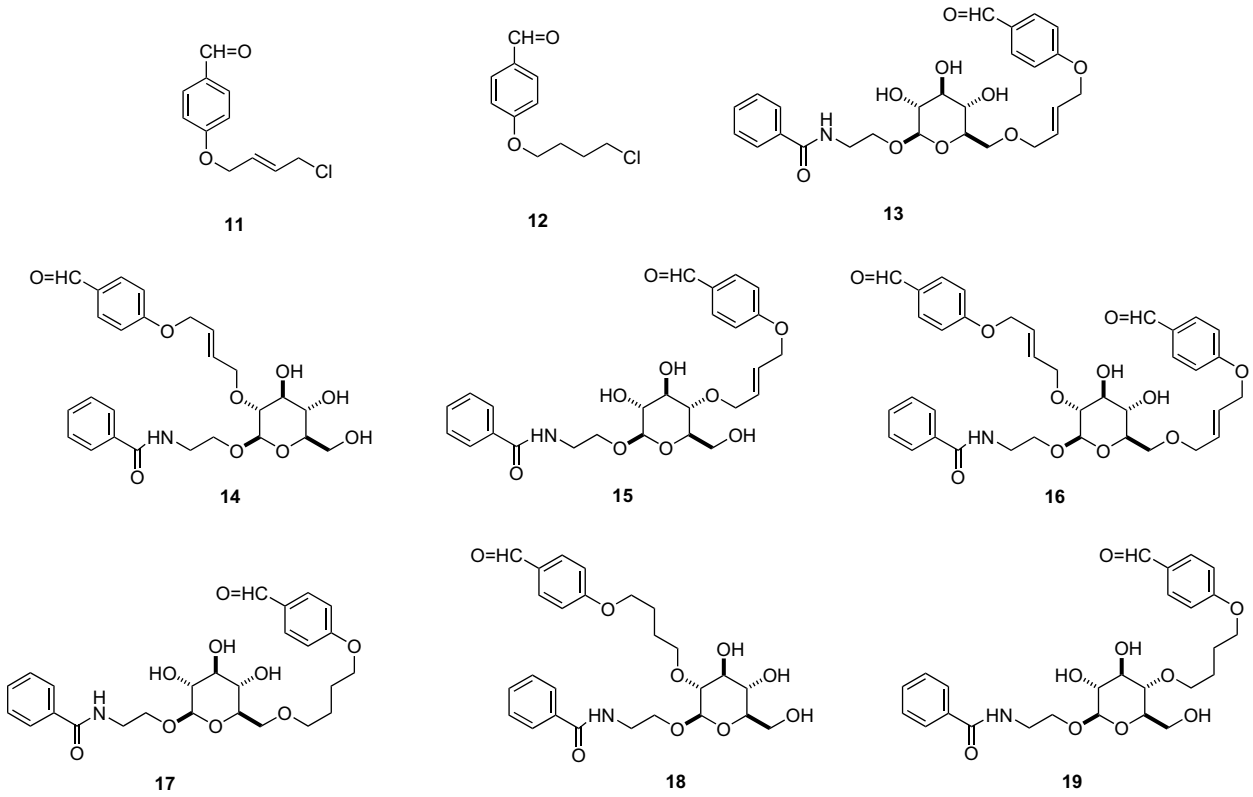


Chart 2.

were discarded because they were not satisfactory for further functionalization. Co-polymers from **Mac8** (**CM8**) were characterized by lamellar structures, not studied further in this context. Co-polymers from **8** (**C8**) showed very attractive spherical shapes with average diameter of 589 nm. These were chosen for a study of functionalization through deprotonation and alkylation of the saccharidic hydroxyl groups.

Model molecules **10** (for the saccharidic moiety) and **11** or **12** (for the electrophilic reagent) were synthesized and used to model the alkylation process. The functionalization of **10**, performed using 10% less than stoichiometric NaH in order to limit the introduction of more than one aldehydic group per saccharidic unit, afforded products of mono or dialkylation involving positions 6, 2 and to a much lesser extent the position 4. Double alkylation was easily avoided by lowering the reaction temperature. Monoalkylation reactions, giving mixtures of 6 and 2-*O*-alkyl derivatives showed a selectivity in favour of the 6-alkylated product, which increased on lowering the reaction temperature.

The functionalization of co-polymer **C8** performed in analogy to **10** with NaH and the more suitable model **11** was confirmed by the fuchsia colour of the Schiff's test, developed on the surface of the solid co-polymer, which was not removable by washing.

4. Experimental

4.1. Materials

1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose (**1**),[18] 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**2**),[19a–c] 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3**),[19b,20] 2-aminoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside toluene-4-sulfonic acid salt (**4**),[21] 4-vinylbenzoyl chloride,[23] 4-vinylbenzoic acid,[26] (*E*)-4-(4-chloro-2-butenyloxy)benzaldehyde (**11**)[11] and 4-(4-chlorobutoxy)benzaldehyde (**12**)[11] were prepared according to known procedures, with the exception of **4**, which was carefully extracted with dry Et_2O to transform the white glassy foam into crystals (mp 150–152 °C, purity 98% by HPLC).

All the reagents including Schiff's fuchsin-sulfite reagent and solvents were purchased from Aldrich. The solvents were dried and distilled according to standard procedures. Petroleum ether refers to the fraction with boiling point 40–60 °C. Styrene (STY), 1,4-divinylbenzene (DVB, containing 80% of DVB isomers) and ethyleneglycol dimethacrylate (EGDM) were distilled at reduced pressure under N_2 and stored at −20 °C. 2,2′-Azobis(2-methylproprionitrile) (AIBN) was crystallized from MeOH.

4.2. Methods

Melting points, determined on a Leica Galen III hot stage apparatus, and boiling points are uncorrected. FTIR spectra were recorded as films or KBr pellets on a Perkin Elmer System 2000 spectrophotometer. ^1H and ^{13}C NMR spectra were acquired on a Bruker Avance DPX 300 Spectrometer at 300 and 75.5 MHz, respectively, using TMS as internal standard and assigned through DEPT-135, COSY, HETCOR and decoupling experiments. GC–MS analyses were obtained with an Ion Trap Varian Saturn 2000 instrument (CI mode, filament current 10 μA) equipped with a DB-5MS (J&W) 30 m, i.d. 0.32 mm, film 1 μm capillary column.

HPLC analyses were performed at rt, constant flow rate (1 mL/min) and UV detection (254 nm) using a Merck LiChrocart 125-4 HPLC cartridge, with a mixture acetonitrile/water=60:40 as eluent.

Flash chromatography (FC) was performed on Merck Silica gel (0.040–0.063 mm). TLCs were obtained on Merck F_{254} silica gel aluminium sheets. The saccharidic compounds were evidenced by spraying the TLC with a 0.3% solution of *o*-aminodiphenyl in H_2SO_4/EtOH (5:95) followed by heating. Preparative layer chromatographies (PLCs) were performed on Merck F_{254} 60 silica gel plates (20×20 cm, 0.5 mm).

Scanning Electron Microscopy (SEM) images were obtained with a Leo Stereoscan 440 instrument (LEO Electron Microscopy Ltd) at Dipartimento di Chimica e Chimica Industriale (Università di Genova). The average diameter of co-polymers **C8** was calculated by measuring directly on the screen the diameters of 41 nanospheres of the SEM image in Figure 2a.

Microanalyses of solid compounds **5**, **7**, **8** and **9** were obtained from the Laboratorio di Microanalisi (Facoltà di Farmacia, Università di Pisa).

4.3. Monomers 5–8

Crystalline **4** (3.0 mmol) in dry CH_2Cl_2 (18 mL) was treated under N_2 at rt with dry Et_3N (1.7 mL) then with acryloyl chloride or 4-vinylbenzoyl chloride[23] (3.0 mmol). After stirring for 4 h at rt the mixture was hydrolyzed with water (18 mL, pH=7–8), extracted with CH_2Cl_2 (3×20 mL) and dried over anhydrous $MgSO_4$. The removal of the solvent at reduced pressure afforded the crude *N*-acryloyl-2-aminoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**5**) as a glassy foam or *N*-(4-vinylbenzoyl)-2-aminoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**7**) as a white solid.

Monomer **5** was purified by two cycles of dissolution in dry THF and precipitation in pentane, dissolution in EtOAc, filtration through a short column of silica gel (h=10 cm, φ=1 cm), evaporation of the solvent and crystallization from Et_2O: yield 67%; mp 114–117 °C, purity 98% by HPLC. FTIR (KBr, ν, cm^{-1}) 3351 (NH), 1759 and 1744 (ester), 1667 (amide), 1634 (C=C), 1066 and 1040 (C–O), 989 (vinyl). ^1H NMR ($CDCl_3$) 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 3.55 (m, 2H), 3.75 (m, 2H), 3.88 (m, 1H), 4.17 (dd, 1H, J=2.5, 12.4 Hz), 4.25 (dd, 1H, J=4.8, 12.4 Hz), 4.51 (d, 1H, J=7.9 Hz), 5.00 (m, 2H), 5.21 (t, 1H, J=9.5 Hz), 5.65 (dd, 1H, J_{cis}=10.2 Hz, J_{gem}=1.6 Hz), 6.11 (dd, 1H, J_{cis}=10.2 Hz, J_{trans}=17.0 Hz), 6.10 (br s, NH), 6.29 (dd, 1H, J_{trans}=17.0 Hz, J_{gem}=1.6 Hz). ^{13}C NMR 20.58, 20.72, 39.25, 61.87, 68.33, 69.30, 71.40, 72.01, 72.61, 101.05, 126.59, 130.74, 165.52, 169.42, 169.55, 170.16, 170.62.

Anal. Calcd for $C_{19}H_{27}NO_{11}$: C, 51.23; H, 6.11; N, 3.14. Found: C, 51.16; H, 6.09; N, 3.13.

Monomer **7** was purified by dissolution in EtOAc (100 mL), filtration through a short column of silica gel (h=13 cm, φ=2 cm), evaporation of the solvent and crystallization in Et_2O: yield 83%; mp 156–157 °C, purity 98% by HPLC. FTIR (KBr, ν, cm^{-1}) 3338 (NH), 1755 (ester), 1649 (amide), 1065 and 1048 (C–O), 992 and 905 (vinyl). ^1H NMR ($CDCl_3$) 1.92 (s, 3H), 2.00 (s, 3H), 2.03 (s, 6H), 3.53–3.90 (m, 4H), 3.96 (m, 1H), 4.10 (dd, 1H, J=2.4, 12.4 Hz), 4.24 (dd, 1H, J=5.0, 12.4 Hz), 4.54 (d, 1H, J=7.9 Hz), 5.05 (m, 2H), 5.21 (t, 1H, J=9.5 Hz), 5.36 (dd, 1H, J_{cis}=10.8 Hz, J_{gem}=0.8 Hz), 5.83 (dd, 1H, J_{trans}=17.6 Hz, J_{gem}=0.8 Hz), 6.60 (br t, NH), 6.74 (dd, 1H, J_{cis}=10.8 Hz, J_{trans}=17.6 Hz), 7.61 (m, 4H). ^{13}C NMR 20.58, 20.66, 39.65, 61.87, 68.34, 69.19, 71.39, 72.00, 72.62, 101.03, 116.00, 126.30, 127.29, 133.34, 135.94, 140.77, 167.02, 169.43, 169.54, 170.15, 170.58. Anal. Calcd for $C_{25}H_{31}NO_{11}$: C, 57.58; H, 5.99; N, 2.69. Found: C, 57.50; H, 6.01; N, 2.68.

The peracetylated monomer (**5** or **7**) (2.0 mmol) was dissolved in dry dioxane (24 mL) and treated with 1.32 M MeONa in MeOH (1.6 mL) at rt for 60 min (the appearance of deacetylated monomers **6** or **8** was observed by TLC using a mixture $CHCl_3$/MeOH=4:1 as eluent) (R_f: **5**=0.79, **6**=0.16, **7**=0.90, **8**=0.40). The dioxane mixture was diluted with MeOH (15 mL) then treated with Amberlite IR120(plus) up to pH=6, filtered and the resin washed with MeOH. Washings and filtrate were combined and evaporated at reduced pressure to afford the crude *N*-acryloyl-2-aminoethyl β-D-glucopyranoside (**6**) or *N*-(4-vinylbenzoyl)-2-aminoethyl β-D-glucopyranoside (**8**) as pink syrups. The syrups were dissolved in a mixture of $CHCl_3$/MeOH 1:1 for **6** or $CHCl_3$/MeOH=4:1 for **8** and passed through a short column of silica gel (h=13 cm, φ=2 cm) using the same mixture of solvents (90 mL) and vacuum evaporated.

A sample of monomer **6** in the form of highly hygroscopic amorphous solid, for characterization purposes was chromatographed by PLC ($CHCl_3$/MeOH 4:1 as eluent) and obtained as a glassy foam.

Compound **6**. Yield 67%. FTIR (KBr, ν, cm^{-1}) 3392 (OH), 1659 (amide), 1627 (C=C), 1077, 1036 (C–O).

^1H NMR (DMSO-d_6) 2.95–3.38 (m, 10H), 4.15 (d, *H*-1, J=7.4 Hz), 4.58 (br s, 1O*H*), 5.03 (br s, 3O*H*), 5.59 (dd, 1H, J_{gem}=2.3 Hz, J_{cis}=10.1 Hz), 6.09 (dd, 1H, J_{gem}=2.3 Hz, J_{cis}=10.1 Hz), 6.28 (dd, 1H, J_{cis}=10.1 Hz, J_{trans}=17.1 Hz), 8.15 (br t, NH). ^{13}C NMR 38.58, 60.97, 67.73, 69.96, 73.33, 76.46, 76.77, 103.05, 125.05, 131.61, 164.63.

The glassy monomer **8** was obtained as a white powder by dissolution in MeOH and precipitation in dry Et_2O.

Compound **8**. Yield 90%. Mp 104–106 °C, purity 98% by HPLC. FTIR (KBr, ν, cm^{-1}) 3351 (OH), 1627 (amide), 1078, 1053 (C–O), 987 and 909 (vinyl). ^1H NMR (DMSO-d_6) 2.94–3.24 (m, 4H), 3.32–3.76 (m, 5H), 3.78–3.94 (m, 1H), 4.20 (d, *H*-1, J=7.7 Hz), 4.54 (t, O*H*, J=5.8 Hz), 4.94 (d, O*H*, J=4.8 Hz), 4.99 (d, O*H*, J=4.5 Hz), 5.07 (d, O*H*, J=4.3 Hz), 5.37 (d, 1H, J_{cis}=11.0 Hz), 5.96 (d, 1H, J_{trans}=17.6 Hz), 6.79 (dd, 1H, J_{cis}=11.0 Hz, J_{trans}=17.6 Hz), 7.70 (m, 4H), 8.44 (t, NH). ^{13}C NMR 39.96, 61.68, 68.20, 70.65, 74.08, 77.20, 77.53, 103.75, 116.77, 126.58, 128.18, 134.17, 136.53, 140.34, 166.61. Anal. Calcd for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.67; H, 6.58; N, 3.95.

4.4. Oligomerizations of monomers 5 and 7

The peracetylated monomer (**5** or **7**) (4.0 mmol), 2-aminoethanethiol hydrochloride (0.8 mmol) and AIBN (4% by weight of the monomer) were dissolved under N_2 in MeOH (20 mL/mmol of monomer **5**) or dry DMF (13 mL/mmol of monomer **7**) in a vial with side arm, subjected to two freeze-pump-thaw cycles at −78 °C, siphoned into the polymerization flask and magnetically stirred at 60 °C for 72–79 h. The mixture was cooled, concentrated under reduced pressure and poured into a large excess of Et_2O (400 mL/g of oligomer). The oligomer was filtered and further purified through two dissolution/precipitation cycles as follows: **Olig5** [MeOH (solvent)/Et_2O (non-solvent)]; **Olig7** [THF (solvent)/Et_2O (non-solvent)] until the disappearance of the residual monomers (**5** or **7**) was observed by TLC using petroleum ether/acetone 1:1 as eluent (R_f: **5**=0.49, **7**=0.73, **Olig5** or **Olig7**=0).

The loading of amino groups in the oligomers in the form of hydrochlorides was determined by dissolving the oligomer (50–80 mg) in THF (1–2 mL), treating the solution with a known excess of 0.1 N NaOH and titrating the unreacted NaOH with 0.1 N HCl.

FTIR spectra of **Olig5** and **Olig7** are characterized by strong absorption bands at 1755 cm^{-1} (ester), 1230 and 1039 cm^{-1} (C–O).

4.5. Macromonomers Mac6 and Mac8

A solution of **Olig5** or **Olig7** (1.0 mmol, as determined by titration of NH_2 group) in dry $CHCl_3$ (55 mL for **Olig5** or 44 mL for **Olig7**), and Et_3N (2.0 mmol) was cooled to 0 °C, treated under N_2 with 4-vinylbenzoyl chloride[23] (1.0 mmol), allowed to reach rt and magnetically stirred until the disappearance of the purple colour developed on TLC after elution, with 0.2% solution of ninhydrin in 95% EtOH. The mixture was then hydrolyzed with water (10 mL), extracted with $CHCl_3$ (3×20 mL) and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the solid macromonomer was purified by repeated dissolution in $CHCl_3$ and precipitation in Et_2O, and brought to constant weight under reduced pressure: **Mac5** (yield 86%); **Mac7** (yield 88%).

A solution of **Mac5** or **Mac7** (0.50 mmol of 4-vinylbenzamido groups) in dry dioxane (35 mL for **Mac5** and 20 mL for **Mac7**) was treated with 1.12 M MeONa in MeOH for 90 min at rt until the disappearance of the peracetylated macromonomer as shown both

by TLC (petroleum ether/acetone=1:1 as eluent) and by the lack of the ester band at 1755 cm^{-1} in the FTIR spectrum. The milky suspension was then treated with MeOH in large excess to favour coagulation and filtered. The solid deacetylated macromonomer was dried and brought to constant weight under reduced pressure: **Mac6** (yield 59%); **Mac8** (yield 91%).

4.6. Precipitation co-polymerizations of Mac8 and 8 with crosslinkers

Monomer **8** or macromonomer **Mac8** or mixture **Mac8**/styrene 1/20.5 w/w and AIBN (Table 1) was dissolved under N_2 with the proper solvent in a vial with side arm, siphoned into the polymerization flask equipped with silicone septum and a Teflon stirring bar, then treated with the crosslinker through a microsyringe. The mixture, after 30 min of N_2 purging, was magnetically stirred at 70 °C for 24–74 h. The milky suspension was centrifuged at 3000 rpm for 30 min to remove the liquid phase and subjected to two cycles of washing/sonication/centrifugation (15 min each) first with THF then Et_2O. The co-polymer was brought to constant weight under reduced pressure (yields 11–27%).

4.7. *N*-Benzoyl-2-aminoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (9)

Compound **4** (2.4258 g, 4.30 mmol) was dissolved under N_2 in dry CH_2Cl_2 (20 mL) and added with dry Et_3N (2.4 mL). After cooling to 0 °C the solution was treated with benzoyl chloride (0.6045 g, 4.30 mmol), stirred at rt for 4 h (until the disappearance of **4**, TLC, petroleum ether/EtOAc=1:2 as eluent), hydrolyzed with water (15 mL, pH=10) and extracted with CH_2Cl_2 (3×15 mL). The extracts were combined, dried over anhydrous $MgSO_4$, concentrated under reduced pressure to afford a dark oil that was passed through a short column of silica gel (h=13 cm, φ=2 cm). The obtained white solid (1.65 g) was crystallized from Et_2O to give **9** (1.39 g, yield 65%). For characterization purposes a sample of **9** was chromatographed by PLC ($CHCl_3$/MeOH=4:1 as eluent, R_f=0.86). Mp 122–123 °C; purity 94% by HPLC. FTIR (KBr, ν, cm^{-1}) 3353 (NH), 1752 (ester), 1648 (amide), 1085 and 1044 (C–O). ^{1}H NMR ($CDCl_3$) 1.91 (s, 3H), 2.00 (s, 3H), 2.03 (s, 6H), 3.54–3.90 (m, 4H), 3.98 (m, 1H), 4.10 (dd, 1H, J=2.3, 12.3 Hz), 4.24 (dd, 1H, J=5.0, 12.3 Hz), 4.55 (d, *H*-1, J=7.9 Hz), 4.98–5.11 (m, 2H), 5.22 (t, 1H, J=9.6 Hz), 6.63 (br s, NH), 7.40–7.56 (m, 3H), 7.78–7.81 (m, 2H). ^{13}C NMR 20.54, 20.58, 20.66, 39.64 (CH_2N), 61.86 (C-6), 68.31 (C-4), 69.18, 71.36 (C-2), 71.97 (C-5), 72.62 (C-3), 101.01 (C-1), 126.95, 128.56, 131.60, 134.32, 167.41, 169.42, 169.51, 170.15, 170.57. Anal. Calcd for $C_{23}H_{29}NO_{11}$: C, 55.75; H, 5.90; N, 2.83. Found: C, 55.78; H, 5.91; N, 2.81.

Compound **9** was also prepared as follows: a mixture of **1** (0.6961 g, 1.8 mmol), *N*-(hydroxyethyl)benzamide (0.3527 g, 2.1 mmol) and CH_2Cl_2 (7 mL) was cooled to 0 °C and treated in the dark with $BF_3{\cdot}OEt_2$ (1.30 g, 9.0 mmol) under stirring for 40 min. The mixture was left at rt for 24 h, then hydrolyzed with water (7 mL). The organic phase was washed in turn with satd $NaHCO_3$, satd NaCl, and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the oily residue was crystallized from a mixture Et_2O/diisopropyl ether (1:1) to give **9** (0.6581 g, yield 75%) having FTIR, ^{1}H and ^{13}C NMR spectra coincident with those reported above.

4.8. *N*-Benzoyl-2-aminoethyl β-D-glucopyranoside (10)

A solution of **9** (0.8099 g, 1.6 mmol) in dry dioxane (20 mL) was treated with 1.32 M MeONa in MeOH (1.2 mL) for 1 h at rt until disappearance of **9** as shown by TLC ($CHCl_3$/MeOH=4:1; R_f: **9**=0.86, **10**=0.29). The solution was diluted with MeOH (5 mL) and treated with Amberlite FTIR120(plus) to neutrality and filtered. The filtrate was concentrated under reduced pressure to afford **10** as an oil that was passed through a short column of silica gel (h=13 cm, φ=2 cm) using a mixture $CHCl_3$/MeOH=4:1 as eluent. The evaporation of the solvent gave a spongy solid that by treatment with dry Et_2O changed to a hygroscopic powder when stored over P_2O_5. Yield 94%. Purity 98% by HPLC. FTIR (KBr, ν, cm^{-1}) 3392 (OH), 1640 (amide), 1077 and 1036 (C–O). ^{1}H NMR (DMSO-d_6) 2.93–3.21 (m, 4H), 3.37–3.75 (m, 5H), 3.84 (m, 1H), 4.19 (d, *H*-1, J=7.7 Hz), 4.54 (t, *OH*, J=5.7 Hz), 4.95 (d, *OH*, J=4.7 Hz), 5.00 (d, *OH*, J=4.3 Hz), 5.08 (d, *OH*, J=3.9 Hz), 7.49 (m, 3H), 7.84 (m, 2H), 8.43 (t, *NH*, J=5.1 Hz). ^{13}C NMR 39.62 (CH_2N), 62.00 (C-6), 68.51, 70.98 (C-4), 74.40 (C-2), 77.53 (C-3), 77.87 (C-5), 104.07 (C-1), 128.12, 129.21, 132.09, 135.36, 167.33.

4.9. Alkylations of 10

4.9.1. General procedure

NaH (60% suspension in white oil) was washed under N_2 with dry pentane (2×2 mL), evaporated, suspended in dry DMF (2 mL), treated with a solution of **10** in dry DMF (2 mL) and magnetically stirred at rt until to get a clear solution (30 min). The clear solution was treated with **11** or **12** under stirring at the desired temperature until no further modifications of the composition of the mixture was evidenced by TLC ($CHCl_3$/MeOH=7:1 as eluent). The mixture was treated with MeOH (2 mL) and evaporated at 60 °C under reduced pressure to give a syrup that was chromatographed by PLC using the same eluent as above (Table 2).

4.10. Functionalization of the nanospherical co-polymer C8

NaH (60% suspension in white oil, 8.0 mg, 0.20 mmol) was washed under N_2 with dry pentane (2×1 mL), vacuum dried, suspended in dry DMF (2.5 mL), added with solid **C8** (50.0 mg, loading of the saccharidic unit 2.83 mmol/g) and magnetically stirred at rt for 30 min. The mixture was treated with **11** (42.0 mg, 0.20 mmol) under stirring at 24 °C for 72 h, added with water (1 mL) and THF (1 mL), centrifuged at 3000 rpm (15 min) to remove the liquid phase and subjected to washing/centrifugation cycles first with THF then Et_2O up to elimination of aldehydic compounds as evidenced by TLC and the negative Schiff's test in the last washings. The solid was brought to constant weight under reduced pressure (13.0 mg) and gave a positive Schiff's test showing an intense fuchsia colour, which after centrifugation of the sample remained on the solid, leaving a colourless liquid phase.

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Supplementary data

Supplementary data concerning the preparation of **Olig5**, **Olig7**, co-polymers of **Mac6** and **Mac8** with styrene, and *N*-(hydroxyethyl)benzamide, ^{1}H, ^{13}C NMR spectra of compounds **5**–**19** can be found in the online version. Supplementary associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.05.033.

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